

PCT/US2003/037666

1. A method for testing a plasmid containing a gene encoding for an endothelial cell mitogen for the ability to produce a biologically active endothelial cell mitogen protein comprising:
 - 5 transiently transfecting a transfection host cell line with a plasmid containing a gene encoding for an endothelial cell mitogen;
 - incubating endothelial cells with conditioned media from the transiently transfected transfection host cell line; and
 - 10 determining the level of cell survival of the endothelial cells incubated with conditioned media from the transfection host cell line transfected with the plasmid containing a gene encoding for an endothelial cell mitogen as compared to endothelial cells incubated with conditioned media from the transfection host cell line transfected with a control plasmid;
 - 15 wherein the level of cell survival of the endothelial cells is determined by the ability of the endothelial cells to reduce MTS to formazan.
2. The method of claim 1, wherein the plasmid contains a gene encoding for an endothelial cell mitogen selected from the group consisting of acidic and basic fibroblast growth factors, vascular endothelial growth factor, epidermal growth factor, transforming growth factor α and β , platelet-derived endothelial growth factor, platelet-derived growth factor, tumor necrosis factor α , hepatocyte growth factor and insulin-like growth factor.
3. The method of claim 2, wherein the plasmid contains a gene encoding for VEGF.
- 25 4. The method of claim 3, wherein the plasmid contains a gene encoding for VEGF A.
- 30 5. The method of claim 3, wherein the plasmid contains a gene encoding for VEGF C.

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6. The method of claim 1, wherein the transfection host cell line is the Cos-1 cell line.

7. The method of claim 1, wherein the endothelial cells are HUVEC cells.
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8. The method of claim 1, wherein the level of cell survival of the endothelial cells incubated with conditioned media from the transfection host cell line transfected with the plasmid containing a gene encoding for an endothelial cell mitogen is at least 25 % fold greater than the level of cell
10 survival of the endothelial cells incubated with conditioned media from the transfection host cell line transfected with the control plasmid.

9. The method of claim 1, wherein the plasmid containing the gene encoding for the endothelial cell mitogen is tested for the ability to produce
15 biologically active endothelial cell mitogen protein prior to use of the plasmid containing the gene encoding for the endothelial cell mitogen in a human gene therapy treatment.

10. A method for evaluating the ability of a first plasmid construct
20 containing a gene encoding for an endothelial cell mitogen to produce a bioactive endothelial cell mitogen protein as compared to the ability of a second plasmid construct containing a gene encoding for an endothelial cell mitogen to produce a bioactive endothelial cell mitogen comprising:

transiently transfecting a transfection host cell line with plasmid DNA
25 containing a gene encoding for an endothelial cell mitogen;

incubating endothelial cells with conditioned media from the transiently transfected transfection host cell line; and

determining the level of cell survival of the endothelial cells incubated with conditioned media from the transfection host cell line transfected with
30 plasmid containing a gene encoding for an endothelial cell mitogen;

wherein the level of cell survival of the endothelial cells is determined by the ability of the endothelial cells to reduce MTS to formazan.

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11. The method of claim 10, wherein the plasmid contains a gene
encoding for an endothelial cell mitogen selected from the group consisting of
acidic and basic fibroblast growth factors, vascular endothelial growth factor,
epidermal growth factor, transforming growth factor α and β , platelet-derived
5 endothelial growth factor, platelet-derived growth factor, tumor necrosis factor
 α , hepatocyte growth factor and insulin-like growth factor.

12. The method of claim 11, wherein the plasmid contains a gene
encoding for VEGF.

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13. The method of claim 12, wherein the plasmid contains a gene
encoding for VEGF A.

14. The method of claim 12, wherein the plasmid contains a gene
15 encoding for VEGF C.

15. The method of claim 10, wherein the transfection host cell line is the
Cos-1 cell line.

20 16. The method of claim 10, wherein the endothelial cells are HUVEC
cells.

17. The method of claim 10, wherein the plasmids containing the gene
encoding for an endothelial cell mitogen are being compared as a means for
25 determining an optimal plasmid construct for use in a human gene therapy
treatment.